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Research Article

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Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of linagliptin and metformin in pure and pharmaceutical dosage form

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ABSTRACT

A simple, RP-HPLC method was established for determining linagliptin and metformin in pharmaceutical formulations. Linagliptin , metformin and their degradation products were separated using C_8 column with Acetonitrile: Water: Methanol (25:50:25 (v/v/v) to pH 4.1 with 0.1% orthophosphoric acid as the mobile phase. Detection was performed at 243 nm using a diode array detector. The method was validated using ICH guidelines and was linear in the range 5-30µg/ and 10-100 µg /ml for linagliptin and metformin respectivily. Good separation of both the analytes and their degradation products was achieved using this method. The developed method can be applied successfully for the determination of linagliptin and metformin.

Keywords: Lingliptin**,** Metformin,Reverse phase liquid chromatography; Validation, Pharmaceutical dosage form

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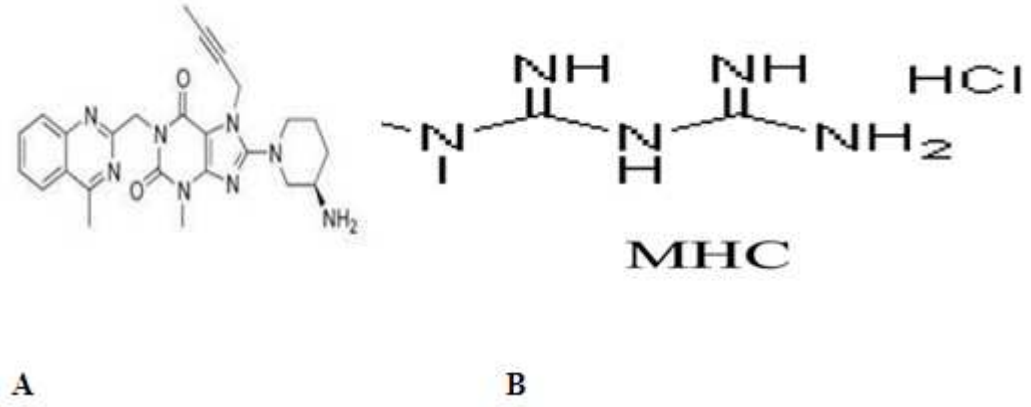
INRODUCTION

Safety and efficacy are two fundamental properties of drug products. Instability of drug products can cause changes in the physical, chemical, pharmacological and toxicological properties of their active pharmaceutical ingredients (API). Therefore pharmacists should take various factors into consideration, for example drug stability, possible degradation products and potential interactions with the excipients used in the formulation, to ensure successful therapy. A stability-indicating procedure is one which, based on the characteristic structural, chemical, or biological properties of each active ingredient of a drug product, will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured[1]. It is recommended by both the ICH and the WHO that analysis of drugs during stability testing should be conducted by use of a validated stability-indicating method. In this paper, ICH and WHO guidelines were therefore heeded for the simultaneous determination of linagliptin and metformin.

Linagliptin is a DPP-4 inhibitor developed by BoehringerIngelheim (German Pharmaceutical Company) for treatment of type II diabetes. Linagliptin was approved by the US FDA on 2 May 2011 for treatment of type II diabetes[2]. It is being marketed by BoehringerIngelheim and Lilly. Linagliptin is an inhibitor of DPP-4 **(**dipeptidyl peptidase 4**)** an enzyme that degrades the incretion hormones, Glucagonlike peptide-1 (GLP-1) and Glucose-dependent Insulinotropic polypeptide (GIP). Both GLP-1 and GIP increase insulin biosynthesis and secretion from pancreatic beta cells in the presence of normal and elevated blood glucose levels. GLP-1 also reduces glucagon secretion from pancreatic alpha cells, resulting in a reduction in hepatic glucose output. Thus, Linagliptin stimulates the release of insulin in a glucose-dependent manner and decreases the levels of glucagon in the circulation. Linagliptin showed that the drug can effectively reduce blood sugar[3].In summary, Linagliptin reduces blood glucose levels by inhibiting DPP-4 and increasing the levels of GLP-1 and GIP. Linagliptin was approved by the FDA in May 2011[4,5].

Metformin hydrochloride (MH) chemically, 3-(diaminomethylidene)-1,1-dimethylguanidine hydrochloride[6]is an antidiabetic agent[7]. It is the drug of choice for the treatment of type II diabetes, particularly in overweight and obese people and individuals with normal kidney function. It works by lowering blood sugar and helping the body use insulin more efficiently. It is available in 500 mg, 850 mg and 1000 mg tablets (immediate release) and in 500 mg and 750 mg (slow release) for oral administration. BoehringerIngelheim Pharmaceuticals, Inc. LP in combination with MH in a single dosage form as Jentadueto™ .[8] In combination these are available in 2.5/500 mg, 2.5/850 mg and 2.5/1000 mg of Linagliptin and MH, respectively.



A B

Fig 1.Structure of Linagliptin(A) and Metformin(B)

The combination of linagliptin and metformin has not been adopted by any official pharmacopoeia. An extensive review of the literature revealed that several methods have been reported forindividually linagliptin and simultaneous estimation of metformin with other drugs and no HPLC method for simultaneous determination of metformin and linagliptin drugs. Therefore attempts were made to develop and validate simple, precise, sensitive and isocratic reverse phase high performance liquid chromatographic method for simultaneous determination of both drugs along with their stress-induced degradation products in pharmaceutical formulations.

EXPERIMENTAL SECTION

Chemicals and reagents

Reference standards of metformin hydrochloride and lignagliptin with stated purity of 99.97 and 99.88%, respectively were obtained from well reputed laboratories. Acetonitrile, Methanol (HPLC grade), Double distilled water was used throughout the analysis. Mobile phase was filtered using 0.45 µm nylon filters by Millipore (USA).

Equipment

The HPLC system consisted of a Shimadzu LC-20A system (Kyoto, Japan) equipped with a model LC-20AT pump, SPD-M10A Diode array detector (set at 243 nm), and a Rheodyne injection valve with a 20 µL loop. Peak areas were integrated using a Shimadzu LC solution (version 1.227) software program. The experimental conditions were optimized on a BDS Hypersil C_8 column (250 × 4.6 mm, 5 µm particle size) at room temperature. Mobile phase consisted of Acetonitrile: Water: Methanol (25:50:25 (v/v/v). The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. Flow rate of the mobile phase was 1.0 ml min^{-1} and all chromatographic experiments were performed at room temperature (25 ºC ± 2 ºC).

Chromatographic Conditions

Chromatographic analysis was performed on aBDS Hypersil C_8 column (250 × 4.6 mm, 5 µm particle size) at room temperature.The mobile phase consists of acetonitrile: water: methanol (25:50:25 (v/v/v) adjusted to pH 4.1 with 0.1% orthophosphoric acid. The flow rate of mobile phase was adjusted to 1ml/min and the injection volume was 20µlIt was filtered through a 0.45 µm nylon membrane and degassed in an ultrasonic bath.Detection was performed at 243nm.

Preparation of standard solution

Standard stock solution was prepared by accurately weighing 100 mg of linagliptin and 100 mg of metformin hydrochloride and then both dissolved in 100 ml of mobile phase. 2 m of the standard stock solution was diluted to 25 m with mobile phase to prepare working standard solution having a concentration of 80 mg μl^{-1} for both lignagliptin and metformin hydrochloride. The solution was filtered through a 0.45 µm nylon filter before analysis.

Preparation of sample solution

The formulation tablets of Linagliptin and metformin (Jentaduetog) were crushed to give finely powdered material. Powder equivalent to 10 mg of drug was taken in 10 ml of volumetric flask containing 5 ml of mobile phase and was shaken to dissolve the drug and then filtered through Ultipor N66 Nylon 6,6 membrane sample filter paper. 2 ml of this solution was then diluted to 25 ml with mobile phase to obtain a concentration of 80µg/ ml for both linagliptin and metformin hydrochloride solution was filtered through a 0.45 mm nylon filter before analysis.

RESULTS AND DISCUSSION

In this work, a simple, sensitive and accurate isocratic RP-HPLC method for simultaneous determination of linagliptin, metformin and their stress-induced degradation products was proposed. Both drugs contain basic nitrogen atoms and therefore have the potential to cause peak tailing due to interactions of these basic nitrogen atoms with the silanol groups of the stationary phase during chromatographic separation. In order to obtain symmetrical peaks with better resolution, the chromatographic conditions i.e. pH of the buffer, concentration of organic modifier and silanol blockers were optimized. Various chromatographic conditions such as mobile phase composition, analytical columns with different packing materials (C_8, C_{18}, phenyl, cyano), and configurations (10, 15, 25 cm columns) were used to obtain sharp peaks with reduced tailing, and better resolution with no peak impurity. Finally, a base deactivated silica end-capped BDS Hypersil C_8 column was selected which provided reduced peak tailing and acceptable peak purity index. Mobile phase composition was selected based upon peak parameters (symmetry, tailing, resolution and peak purity index etc.), run time, ease of preparation and cost. The most suitable mobile phase composition was found to be Acetonitrile: Water: Methanol (25:50:25 (v/v/v) to pH 4.1 with 0.1% Orthophosphoric acid. Under the chromatographic conditions outlined, highly symmetrical and sharp peaks of metforminand linagliptinwere obtained at retention times of 2.90 and 7.00mins, respectively (Figure 1).

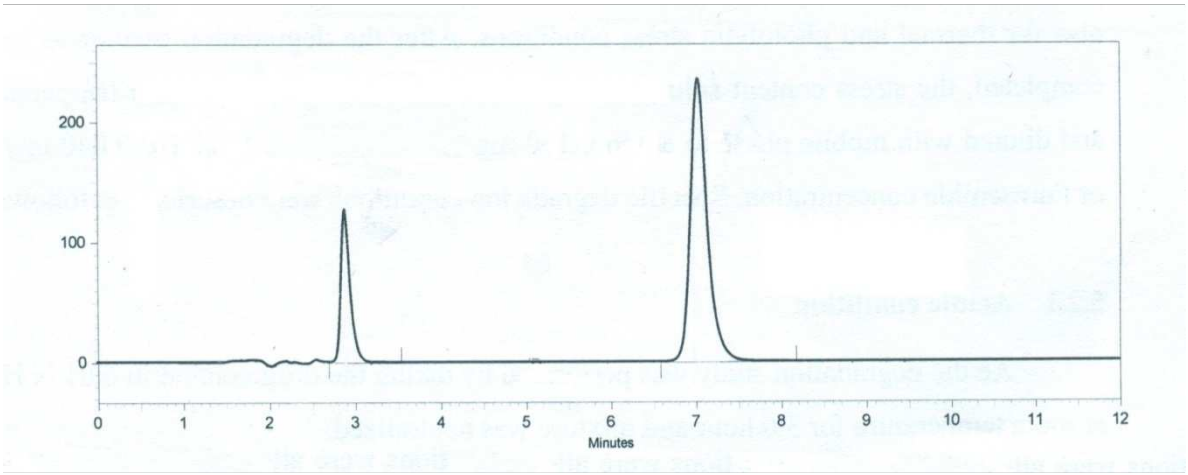


Fig 1. Chromatogram of metformin(2.90 min) and linagliptin(7.00 min)

Method Validation

The chromatographic method developed was validated using ICH guidelines[9,10]. Validation parameters include linearity, accuracy, precision, robustness, specificity, limit of detection and quantitation.

Linearity

Linear calibration plots of the proposed method were obtained over concentration ranges of 5-30 µg ml for both linagliptin and metformin hydrochloride 10-100 µg ml. Each solution was prepared in triplicate.The linear regression equation for linagliptin was found to be Y= 64609 X + 868381 whereas for metformin was Y= 37793 X + 247563. The correlation coefficient for metformin and linagliptin are 0.9989 and 0.9983 respectively. The results of the regression statistics of linagliptin and metformin are given in Table 1. The residual plots of linagliptin and metformin are shown in Figures 2 and 3 .

Table1 Linear regression equations and correlation coefficient

Component	Range**(µg /ml)**	**Slope**	**Intercept**	**Correlation coefficient**
Linagliptin	5-25	64609	868381	0.9989
Metformin	10-50	37793	247563	0.9983

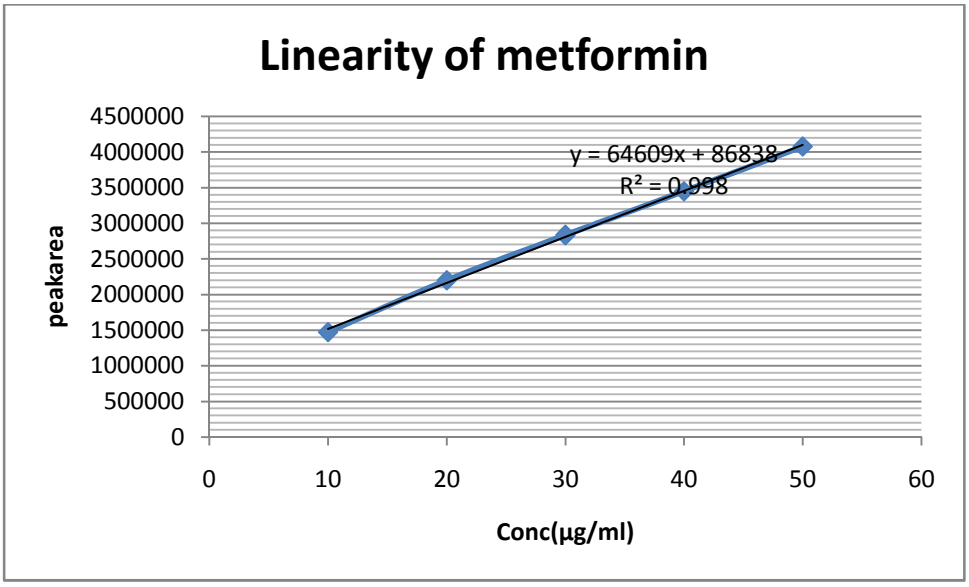


Fig 2 Linearity graph for metformin

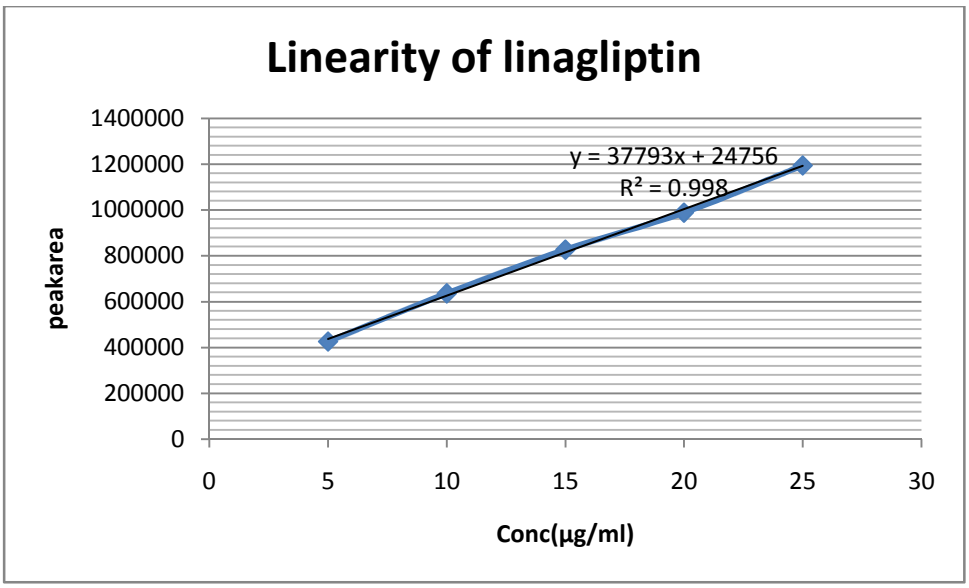


Fig 3 Linearity graph for linagliptin

Accuracy

Accuracy of the method was determined by the standard addition technique. Three levels of solutions (50, 100 and 150%) of the nominal analytical concentrations were prepared. Percentage recoveries, along with standard deviation and relative standard deviations for each analyte (n = 5) are given in Table 2. From the data given in Table 2, it is clear that the method is highly accurate and suitable for the intended use.

Table2 Accuracy of the proposed method

Component	Level (%)	Theoretical concentration(µg/ml)	Observed concentration (µg/ml)±SD,RSD%	Recovery%	%RSD
Linagliptin	50	2.5	2.46	99.76	0.49
	100	10	10.23	101.098	1.68
	150	15	15.67	101.71	1.38
Metformin	50	250	249.98	100	0.32
	100	500	498.90	99	1.23
	150	750	749.98	100	1.37

Precision

Intra-day precision was determined by injecting 5 standard solutions of 3 different concentrations on the same day (n = 5) whereas inter-day precision was determined by injecting the same solutions for 3 consecutive days. Relative standard deviation (RSD %) of the peak area was calculated to represent precision. The results of intra-day and inter-day precision are shown in Table 3. RSD values were less than 2% for intra and inter day precisions, indicating high precision of the method.

Table 3.Intaday and interday precision of the proposed method

Component	Actual concentration(µg/ml)	Intra day Measured concentration (µg/ml)±SD,RSD%	Intra day Measured concentration (µg/ml)±SD,RSD%
Linagliptin	2.5	2.45±0.7;1.2	2.38±0.9;1.6
	5.0	4.90±1.5;1.2	4.84±0.9:1.5
	7.5	7.47±0.9;0.9	7.29±1.8;1.2
Metformin	250	250.59±0.4;0.9	250.78±0.8;1.4
	500	547.71±0.3;0.4	548.29±1.0;1.3
	750	748.6±0.3;0.7	748.09±1.4;1.2

Specificity (stress testing)

The specificity of the method was determined by checking the interference of placebo with analyteand the proposed method were eluted by checking the peak purity of linagliptin and metformin during the force degradation study. The peak purity of the linagliptin and metformin were found satisfactory under different stress condition. There was no interference of any peak of degradation product with drug peak.Stress testing was carried out using different ICH prescribed stress conditions such as acidic, basic, oxidative, photolytic and thermal stresses. The drug content were found to be degrading up to 9%,6%,0.35% in acidic, alkaline and oxidative condition respectively. In thermal and photolytic condition the drugs

Robustness

Robustness of the method was ascertained by slightly varying the chromatographic conditions. The results showed that slight variations in chromatographic conditions had negligible effect on the chromatographic parameters. The results of the robustness study are given in Table 4&5

Table 4 Robustness study of linagliptin

Component	Chromatographic conditions	Range	Assay	Theoretical plates	Tailing	Rt
Linagliptin	Mobile phase (ACN: Water: Methanol) (v/v/v)	15:70:15	100.82	5518	1.17	5.47
		25:50:25	99.56	5591	1.13	5.27
		10:80:10	110.25	5522	1.18	5.12
	Flow rate(ml/min)	0.6	100.75	5512	1.22	6.09
		0.8	99.39	5580	1.32	4.7
	pH	3.5	99.59	5511	1.17	5.1
		2.5	99.36	5537	1.18	5.3

Table 5 Robustness study of metformin

Component	Chromatographic conditions	Range	Assay	Theoretical plates	Tailing	Rt
Metformin	Mobile phase (ACN: Water: Methanol) (v/v/v)	15:70:15	100.08	6890	1.11	11.931
		25:50:25	100.71	6890	1.02	11.491
		10:80:10	100.22	6890	1.20	11.143
	Flow rate(ml/min)	0.6	99.91	68.21	1.11	13.32
		0.8	100.49	68.70	1.19	10.08
	pH	3.5	99.66	6861	1.17	11.32
		2.5	99.096850	6899	1.11	11.56

Limit of detection and limit of quantitation

The limits of detection (LOD) and quantitation (LOQ) were determined by making serials of dilutions LOD and LOQ values show in the table 6 that the method can be used for analysis of these drugs at very low concentrations, which in some cases during stability studies is very challenging.

Table 6 LOD,LOQ data for linagliptinand metformin

Component	LOQ(mcg/ml)	LOD(mcg/mg)
Linagliptin	0.15	0.09
metformin	0.15	0.06

CONCLUSION

A simple, sensitive, and accurate method using reverse phase HPLC was described for simultaneous determination of linagliptin and metformin hydrochloride in pharmaceutical formulations. The proposed method was validated by testing its linearity, accuracy, precision, limits of detection, and quantitation, and specificity. The method proved able to separate the peaks of active pharmaceutical ingredients (APIs) from the degradation products (produced during forced degradation studies). It is also clear from the chromatograms that both the active ingredient peaks under all the stress conditions were free from any sort of degradation impurities. Taken together, these results allow us to conclude that the method can be successfully used for all stability and validation studies.

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